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Optimization of sugar recovery efficiency using microwave assisted alkaline pretreatment of cassava stem using response surface methodology and its structural characterization

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ABSTRACT

Cassava stem is one of the prominent lignocellulosic wastes and has potential as a feedstock for fermentable sugar production. In this study, response surface methodology (RSM) with Box-Behnken design (BBD) was employed to investigate optimum conditions for microwave assisted alkaline pretreatment of cassava stem. Effect of four variables such as reaction time (60–120 s), NaOH concentration (2–4% w/v), solid to liquid ratio (1:25–1:75 g/ml), and microwave frequency (360–720 Hz) were evaluated to improve the sugar recovery. The quadratic model indicated that, reaction time of 116.4 s, NaOH concentration of 3.21% (w/v), substrate to liquid ratio of 1:62.07 g/ml and microwave frequency of 719.86 Hz was found to be optimum and obtained a maximum yield of 43.60 μg/ml of reducing sugar and 91.71 μg/ml of xylose. Under this condition, the cellulose content of cassava stem was increased from 33.27% to 52.34%, while the hemicellulose and lignin content was decreased from 32.30% to 27.15% and 27.15% 14.59%, respectively. Moreover, to evaluate the effectiveness of the pretreatment, Fourier transform infrared (FTIR) spectroscopy, X-ray diffraction (XRD) and scanning electron microscope (SEM) analysis were employed on the untreated and pretreated cassava stem. These results suggest that the microwave assisted alkaline NaOH pretreatment (MAASHP) influences the fermentable sugar production significantly and further it can be utilized effectively for bioethanol production.

1. Introduction

Lignocellulosic materials and agricultural residues are the most attractive and abundant renewable biomass source available in nature. These materials constitute three main polymers namely cellulose, hemicellulose and lignin [1,2]. Cassava (*Manihot esculenta* Crantz) is an everlasting woody shrub belongs to the family *Euphorbiaceae* grows well in tropical and subtropical areas and is generally regarded as a third largest carbohydrate source [3]. It is mainly used ad food (48%) and feed (34%), feedstock (18%) for biofuels and biochemical [4,5]. Globally, cassava stem waste have today reached about 6.7 Pg; 60% of this is available for biofuel production, yielding ca. 64.8 EJ/of energy (given the net heat value of 16.2 MJ/kg). This reported value equals the annual energy use of about 840 million people based on the global average of energy use per capita [6]. A detailed phytochemistry report and a case study on above ground carbon stocks of cassava were found elsewhere in the world. Previous studies reported that non-edible parts of cassava especially stem can be feasibly utilized for fermentable sugars and bioethanol production [7,8].

The major advantage of using non-edible part is neglecting food versus fuel conflict and non-compete with the food supply. To increase the yield of fermentable sugars, an efficient pretreatment method should be incorporated into the lignocellulosic conversion process. Various researchers have reported that pretreatment methods are able to change the efficient lignocellulosic conversion and enhance the biofuel production [7,9,10]. Among the methods, alkaline pretreatment was shown to be more effective and advantageous since it operates at lower temperatures [2] utilizing readily available low-cost chemicals such as sodium hydroxide (NaOH), calcium hydroxide ($Ca(OH)_2$), potassium hydroxide (KOH) and ammonia. However, long retention time and neutralizing pretreated slurry fetch drawbacks to this method. Application of microwaves on pretreatment of lignocellulosic has been considered as an alternative to conventional methods [11,12]. Microwave, a high-frequency electromagnetic radiation offers uniform and rapid heating leading to improved digestibility and disruption of recalcitrant structures in biomass [13].

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Various researchers on several different lignocellulosic feedstocks such as switch grass, wheat straw, water hyacinth, banana waste, sugarcane bagasse, catalpa sawdust, are canut husk [1,3,7,14–18], and so on. Overview of fundamentals, recent advances in microwave assisted pretreatment methods and the importance of microwave reactor development was also available in the literature [11,19].

The present study involves the optimization of MAASHP using cassava stem powder as raw material to increase sugar recovery using response surface methodology. This study aimed to develop the model using cassava stem powder as output and reaction time, NaOH concentration, solid to liquid ratio and microwave frequency as input. The parameters influencing pretreatment conditions were statistically optimized to enhance sugar production. FTIR, XRD and SEM were investigated for determination of functional group modifications, structure of cassava stem and cellulose crystallinity.

2. Materials and methods

2.1. Sample collection and preparation

Lignocellulosic biomass is a substrate for the production of bioethanol was cassava stem from a local farm in Namakkal District (latitude 11.378476; longitude 77.894493), Tamil Nadu, India and the collected material was stored at room temperature for their further analysis. The procured sample was initially shredded into small pieces and then followed by grinding using mixer grinder (Preethi Trio, India). The resultant was sieved using a standard mesh to get fine particles (≤1 mm) and dehydrated in a hot air oven at 65 °C to reach the equilibrium moisture content and later it was stored in a closed container at room temperature (37 °C) for further use. For this experiment, all chemicals and reagents were of analytical grade.

2.2. Pretreatment method

MAASHP was carried out using a domestic microwave oven (LG Model MS-267T). RSM using BBD was used to determine the effect of four different process variables such as reaction time (60, 90, 120 s), NaOH concentration (2, 3, 4% w/v), solid to liquid ratio (1:25, 1:50, 1:75 g/ml), microwave frequency (360, 540, 720 Hz) on the yield of TRS and xylose (μg/ml). The pretreatment experiments were carried out by adding 1 g of the sample with a calculated amount of NaOH solution in 100 mL conical flask forming biomass slurry. Moreover, each run also maintains mentioned solid to liquid ratio. The temperature was not monitored during the experiments since it's abruptly changed. The actual levels of four independent variables are listed in Table 1.

After pretreatment, the samples were cooled and the pretreated slurry was filtered through Buchner funnel to separate the solid and liquid fraction. The collected liquid fraction was neutralized to pH 7 ± 0.2 using concentrated hydrochloric acid (0.1 N HCl). The total reducing sugar using DNS method and xylose content using Orcinol method [20,21] was determined on the liquid fraction. However, the solid fraction was washed several times with distilled water until the wash flow becomes neutral and dried for further analysis.

Table 1
Factors and their levels for BBD.

Variable	Unit	Symbol	Range and their levels		
			−1	0	+1
Reaction time	sec	X_1	60	90	120
NaOH concentration	%	X_2	2	3	4
Solid to liquid ratio	gm/ml	X_3	1:25	1:50	1:75
Microwave Frequency	Hz	X_4	360	540	720

2.3. Design of experiments

Selection of factorial levels was based on preliminary lab experiments done. The four pretreatment factors were chosen as X_1, X_2, X_3, and X_4 which set into three levels coded as −1, 0 and +1. The experimental design consists of 30 experimental runs and these experiments enable one to determine the effect of each independent process variables (reaction time, NaOH concentration, solid to liquid ratio, and microwave frequency) and the interaction between these variables on the dependent variable (TRS and xylose). A software Design Expert 10.0.6 version (Stat Ease Inc., Minneapolis, MN, USA) was employed to perform analysis of variance (ANOVA) and to generate response surfaces. The experimental data were evaluated using response surface regression which is given by the following polynomial.

$$Y_{TRS} \text{ or } Y_{xylose} = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{14} X_1 X_4 + \beta_{23} X_2 X_3 + \beta_{24} X_2 X_4 + \beta_{34} X_3 X_4 + \beta_{11} {X_1}^2 + \beta_{22} {X_2}^2 + \beta_{33} {X_3}^2 + \beta_{44} {X_4}^2 \quad (1)$$

From Eq. (1), Y_{TRS} or Y_{xylose} is the predicted response variable of TRA and xylose; X_1, X_2, X_3, and X_4 are the independent process variables; β_1, β_2, β_3, and β_4 are the linear coefficients; β_{12}, β_{13}, β_{14}, β_{23}, β_{24}, and β_{24} are interaction coefficients and β_{11}, β_{22}, β_{33}, and β_{44} are second order or quadratic coefficients. Response surface and contour plots were drawn using the fitted quadratic polynomial equation obtained from the regression equation.

2.4. Characterization studies

Cellulose content was determined using the acetic/nitric reagent method [22] and the total carbohydrate content (holocellulose) of cassava stem was determined by sodium chlorite-acetic acid method [23]. Lignin content was determined [24]. Ash content was estimated by heating the sample in a muffle furnace at 600 °C for 4 h. The resultant residue was cooled and weighed gravimetrically. All the biochemical characterization was done on dry-weight basis.

Extractives present in the sample were estimated as in Eq. (2).

$$\text{Extractives } (\%) = 100 - (\text{Cellulose } (\%) + \text{Hemicellulose } (\%) + \text{Lignin } (\%) + \text{Ash } (\%)) \quad (2)$$

The hemicellulose in the sample was estimated as in Eq. (3).

$$\text{Hemicellulose } (\%) = \text{Holocellulose } (\%) - \text{Cellulose } (\%) \quad (3)$$

The dried treated and untreated samples were investigated using FTIR analysis and samples were pressed into a disc with KBr. The chemical structure and functional group modifications were analyzed using FTIR spectrophotometer (Shimadzu IRAffinity-1S). The crystalline and amorphous nature of untreated and pretreated cassava stem samples was studied using X-ray diffractometer (XRD, PANalytical XPERT-3) using Cu as anode material with generator settings of 30 mA applied potential and 45 kV current. The samples were measured at a temperature of 25 °C and were scanned in the 2θ range starting at 10° and ending at 90°with a step size of 0.013°. The crystallinity index (CrI) was determined from the relationship between the intensity of cellulose (I_{002}) peak at 22.63° and the minimum dip (I_{am}) peaks at 18.00°, 2θ degrees using the equation mentioned in Eq. (4).

$$CrI(\%) = \frac{I_{002} - I_{am}}{I_{002}} \times 100 \quad (4)$$

where I_{002} is the maximum diffraction intensity of the 002 lattice diffraction and I_{am} is the diffraction intensity of the amorphous portion [25]. Physical changes and surface characteristics of the cassava sample